GERM TUBE MORPHOLOGY OF CANDIDA SPECIES WITH ANTINEOPLASTIC AGENTS IN HUMAN SERUM

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Increased incidence of Candida yeast infection contributing to the death of cancer patients has been noted, with total fungal infections as high as 25% of patients with malignant diseases (1). In order to suppress the rapid growth of neoplastic cells, antitumor agents acted cytotoxically to disrupt protein synthesis in rapidly dividing cells which would lead to cell death (2). At times these agents are responsible for life threatening infection in patients, while others are capable of inducing mutagenesis and oncogenesis in experimental animals (3), and mutations in clinical isolates of Candida albicans in an artificial medium (4).

In this study, variation in germ tube production of pathogenic Candida species in human serum containing antineoplastic agents was examined. Nine chemotherapy drugs used in treatment of various neoplasms were selected for study.

Materials and Methods

Candida albicans QC 31, C. albicans B 344, and C. stellatoidea 445 were isolated from patients with systemic candidosis at Saint Joseph Mercy Hospital, Ann Arbor, MI. The strains had a high rate of germ tube formation in human blood serum. Stock cultures of the isolates maintained on Sabouraud dextrose agar (SDA) were transferred to fresh agar slants monthly and were examined for sugar fermentation patterns, chlamydospore production on cornmeal Tween 80 agar (CTA), and consistency of morphological characteristics in human serum (5).

Yeast cells of Candida species were prepared in shake culture (6) using the following growth medium per liter: glucose 15 g; KH_2PO_4, 2 g; $(NH_4)_2SO_4$, 0.5 g; $CaCl_2 \cdot 2H_2O$, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.05 g; final pH 5.0. The medium was used without the recommended biotin which induces mycelial formation in Candida sp. (7).

Inocula were prepared by transferring a loopful of yeast cells from stock cultures to fresh SDA slants and incubated at 37 C for 24 h. A loopful of the 24 h old yeast cells was inoculated into 250 ml growth medium using 1000 ml Erlenmeyer flasks. The inoculated flasks were placed in a rotary shaker at room temperature, and rotated at 180 rpm. Cells were harvested after 24 h and washed 3 times by centrifuging in distilled water. Collected cells were then resuspended in 20 ml of 0.05 M phosphate buffer (pH 5.5) and stored at 4 C. Cell morphology of this suspension was the yeast form.

All drugs were individually dissolved in sterile human blood serum at concentrations of 5 mg/ml. Drug solutions were prepared and used within 24 h. All drugs were pharmaceutical grade except for 6-mercaptopurine. Stock solutions were diluted in serum as needed to concentrations appropriate for each experiment. All drugs were screened for their ability to inhibit germ tubes as well as

their fungistatic and fungicidal properties through interaction with the viable yeast cells.

Collected yeast cells were washed and resuspended in distilled water to give a stock concentration of 2×10^8 cells/ml. The stock suspension was added to each drug human serum solution for a final concentration of 1×10^4 cells/ml. Percentage germ tube production was determined at 37 C, 3 and 5 h incubation. A 0.1 ml volume of each lightly vortexed cell suspension was placed in a Howard Mold Counting Chamber and examined for germ tube cell counts.

To determine fungistatic activity of test drugs, yeast cells of each *Candida* strain were incubated in drug serum solutions for 24 h. Serum incubated *Candida* cells served as the controls. One ml of each culture was plated on gentamicin supplemented CTA plates, incubated at room temperature, and examined daily for one week for yeast morphological characteristics. Data collected represent the mean of 5-10 replications of each experiment.

Results

Candida germ tube inhibition occurred with nitrogen mustard and doxorubicin at 0.1 mg/ml, and 5-fluorouracyl at 3 mg/ml in human serum at 3 h incubation. Other compounds tested showed no effect at low dose levels (Table I). Drugs at high dose levels produced noted effects on *Candida* cells (Table II). In serum, nitrogen mustard lysed all yeast cells at 2 mg/ml. Fungistatic activity occurred with 5-fluorouracyl at 5 mg/ml, methotrexate at 2.5 mg/ml, and bleomycin and doxorubicin at 2 mg/ml. Cyclophosphamide, 6-mercaptopurine, vinblastine, and vincristine produced no effects on germ tube production at tested concentrations.

On CTA after 24 h incubation, pseudohyphae, blastospores, and chlamydospores appeared with the respective *Candida* controls. After 24 h exposure to drugs at low dose levels, only *Candida* isolates incubated in nitrogen mustard were delayed in forming pseudomycelia. Other drug incubated strains appeared to have pseudohyphae and chlamydospore production at the same time as the controls. Yeast cells removed from the nitrogen mustard serum at the high dose level produced no growth. Chlamydosporulation and filament growth in *C. albicans* B 344 were delayed 2 days with 5-fluorouracyl in serum while other *Candida* isolates exposed 24 h to 5-fluorouacyl in serum then subcultured on CTA only produced yeast colonies without pseudomycelium during the 4 day incubation period. Yeast cells exposed to the other drugs and the controls produced normal chlamydospore and pseudomycelial growth when transferred to the CTA.

Discussion

The 9 drugs assayed included alkylating agents affecting DNA synthesis; analogs of folic acid, pyrimidines, and purines acting as antimetabolites; and natural products interfering with metabolic pathways and mitotic processes (2,3). *Candida* isolates have been shown in other studies to be effected by selected drugs at cell division, growth, and viability (8,9). Drugs which prevented germ tube formation in the current study were agents that interfered first with DNA synthesis and to a lesser extent with RNA and protein synthesis, although germ tube production appears to be

dependent on mitochondrial RNA polymerase activity (10).

The alkylating agent, nitrogen mustard, demonstrated ability to arrest germ tube emergence at low concentrations (Table I) and capability to lyse the cells at higher concentrations (Table II). Doses of cyclophosphamide higher than those of nitrogen mustard were required to achieve any inhibition. The mechanism of action of the 2 drugs is that they are thought to alkylate the purine base guanine, which is found in high quantities in yeast cells (8), resulting in abnormal growth and cell lysis (3). The drug 5-fluorouracyl has been shown to be incorporated into RNA and DNA in C. albicans presumably contributing to the cytotoxic properties when used at high concentrations (11). As a result, germ tube and pseudomycelial production was reduced. Bleomycin, doxorubicin, and 6-mercaptopurine interfering with DNA synthesis did not suppress germ tube emergence at low concentrations.

Two natural products, vinblastine and vincristine, did not suppress germ tube growth of C. albicans QC 31, C. albicans B 344, and C. stellatoidea 445. Methotrexate is a folic acid analog which intervenes with the enzyme dihydrofolate reductase in cells (3). The growth of C. albicans is temporarily repressed at the level of folate activity by any folic acid analog (12), but methotrexate produced no growth change with Candida species in the present work.

At the level of cell viability, none of the drugs produced fungicidal effects, except nitrogen mustard. Land et al. (4) found minimum inhibitory concentrations of germ tube production with drugs such as vincristine, vinblastine, bleomycin, doxorubicin, and 5-fluorouracyl in studies using a synthetic medium substitute for serum. The synthetic medium was shown earlier to induce germ tube development in C. albicans (13,14). Variation to drug sensitivity in synthetic media and human sera could be due to differences in Candida strains used in the various studies. However, a defined medium does not reconstitute the complex composition of nutrients to which C. albicans strains were exposed in human serum (15). Chemotherapy drugs may also alter human serum to allow variation in germ tube production in species of Candida.

Summary

Yeast cells of Candida species were inoculated into human serum containing cancer chemotherapy agents at levels administered to patients. Nitrogen mustard, 5-fluorouracyl, and doxorubicin inhibited yeast germ tube production while other antineoplastic agents had no effect on the cell morphology.

Acknowledgements

We thank Drs. T. J. Barry, E. S. Beneke, E. M. Britt, and A. L. Rogers for yeast isolates and human sera, Lilly Research Laboratories for vinblastine and vincristine, St. Joseph Mercy Hospital Ann Arbor and Beyer Memorial Hospital Ypsilanti for clinical assistance.

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Table I % Germ tube production in human serum with drugs at low dose levels.

Organism	Drug concentration							(mg/ml)		
	SR	5FU	MX	DR	BL	CP	6MP	NM	VB	VC
	0	3	0.25	0.1	0.1	0.12	0.06	0.1	0.06	0.03
				3 hour incubation						
CaQC31 (%)	80	60	80	60	80	80	80	10	80	80
CaB344	80	50	70	40	60	80	80	00	80	80
Cs455	70	40	70	20	40	60	70	00	60	60
				5 hour incubation						
CaQC31	100	80	100	100	100	100	100	20	100	100
CaB344	100	80	100	100	100	100	100	10	100	100
Cs445	100	80	100	100	100	100	100	10	100	100

Table II % Germ tube production in human serum with drugs at high dose levels.

Organism	Drug concentration							(mg/ml)		
	SR	5FU	MX	DR	BL	CP	6MP	NM	VB	VC
	0	5	2.5	2	2	4	4	2	2	2
				3 hour incubation						
CaQC31 (%)	80	30	60	10	50	80	80	00	80	80
CaB344	80	30	60	10	50	80	80	00	70	80
Cs445	80	20	50	20	60	80	80	00	70	80
				5 hour incubation						
CaQC31 (%)	100	70	100	30	70	100	100	00	100	100
CaB344	100	70	100	30	80	100	100	00	100	100
Cs445	100	80	100	40	80	100	100	00	100	100

SR: Serum
5FU: 5-Fluorouracyl
MX: Methotrexate
DR: Doxorubicin
BL: Bleomycin
CP: Cyclophosphamide
6MP: 6-Mercaptopurine
NM: Nitrogen mustard
VB: Vinblastine
VC: Vincristine

CaQC31: *Candida albicans* QC 31
CaB344: *Candida albicans* B 344
Cs445: *Candida stellatoidea* 445